An intermittent hypercaloric diet alters gut microbiota, prefrontal cortical gene expression and social behaviours in rats

Amy C Reichelt *, Amy Loughman, Ashton Bernard, Mukesh Raipuria, Kirsten N Abbott, James Dachtler, Thi Thu Hao Van, Robert J Moore

* Corresponding Author

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Abstract

Objectives: Excessive consumption of high fat and high sugar (HFHS) diets alters reward processing, behaviour, and changes gut microbiota profiles. Previous studies in gnotobiotic mice also provide evidence that these gut microorganisms may influence social behaviour. To further investigate these interactions, we examined the impact of the intermittent access to a HFHS diet on social behaviour, gene expression and microbiota composition in adolescent rats.

Methods: Male rats were permitted intermittent daily access (2h / day) to a palatable HFHS chow diet for 28 days across adolescence. Social interaction, social memory and novel object recognition were assessed during this period. Following testing, RT-PCR was conducted on hippocampal and prefrontal cortex (PFC) samples. 16S ribosomal RNA amplicon sequencing was used for identification and relative quantification of bacterial taxa in faecal samples.

Results: We observed reduced social interaction behaviours, impaired social memory and novel object recognition in HFHS diet rats compared to chow controls. RT-PCR revealed reduced levels of monoamine oxidase A (*Maoa*), catechol-O-methyltransferase (*Comt*) and brain derived neurotrophic factor (*Bdnf*) mRNA in the PFC of HFHS diet rats. Faecal microbiota analysis demonstrated that the relative abundance of a number of specific bacterial taxa differed significantly between the two diet groups, in particular, *Lachnospiraceae* and *Ruminoccoceae* bacteria.

Discussion: Intermittent HFHS diet consumption evoked physiological changes to the brain, particularly expression of mRNA associated with reward and neuroplasticity, and gut microbiome. These changes may underpin the observed alterations to social behaviours.

1. Introduction

The global rate of obesity is rapidly growing, and it is of great concern that the incidence of overweight and obesity is increasing amongst young people and children [1], who most frequently consume hypercaloric high fat and high sucrose (HFHS) 'junk' foods [2]. Studies in rodents have indicated that chronic exposure to hypercaloric diets causes multiple changes to behavioural processes and reward systems, including decreased dopamine turnover in the mesolimbic system [3]. The effects of chronic HFHS diet consumption may be particularly pronounced during critical windows of neurodevelopment. This is supported by emerging data indicating that adolescence may be a sensitive period for susceptibility to diet-induced behavioural changes in mood [4], reward seeking [5, 6] and cognition [7].

Beyond a role in cognition, recent studies have suggested that hypercaloric diet-induced obesity may evoke changes to social behaviour in rodents [8-10]. High fat diet consumption increased social interaction in adult male mice, but impaired recognition memory for a novel *versus* familiar mouse [11], and social recognition is reduced in juvenile rats following short term exposure to high fat diets [10]. Social play, a characteristic adolescent social behaviour in rats that decreases into adulthood [12], was shown to be reduced following neonatal overfeeding, suggesting that early-life nutrition may impact the expression of this behaviour in rats [8]. However, the litter size manipulation utilised in neonatal rodent overfeeding protocols may have also contributed to the altered social repertoires observed.

Previous research has demonstrated overlapping neuronal substrates supporting social behaviour and those that are altered by HFHS diet. Maturation of the prefrontal cortex (PFC) throughout adolescence [13] represents a critical period of vulnerability to diet-evoked

cognitive deficits [14]. The PFC has a critical role in social processing [15, 16], and the appropriate maturation of this region is fundamental for the development of social cognition [17]. Further experimental evidence highlights that the rodent homologue of the medial PFC and the hippocampus are important for social behaviour, including social memory and sociability [18-20]. As aspects of social interaction are rewarding, it is proposed that the increased dopamine efflux and ongoing refinement of reward-associated neural connections within the PFC across adolescence accentuate this behaviour in young rats [12]

Previous studies have highlighted that dysfunction in the PFC is induced through intermittent access to a HFHS diet [21], or a continuously-available high fat diet [7] during adolescence, supporting evidence that PFC neuropathology underpins social deficits [22]. In particular, intermittent access to palatable foods has been shown to impact on reward neurocircuitry [23, 24], and furthermore allows examination of behaviour both immediately following palatable food consumption, and when animals have not had recent access to the same palatable food source.

Moreover, dietary manipulations also influence gut microbial composition [25], and alterations to gut flora has been linked to changes in cognition, mood and behaviour [26, 27]. Studies utilising germ-free (GF) mice demonstrated that the presence, composition, and functionality of the gut microbiota is crucial for normal social behaviours, which are reduced in GF mice [26]. GF mice and antibiotic-induced gut dysbiosis rodent models have demonstrated associations between the disruption of the gut microbial community and cognitive, social and emotional alterations [26, 27].

Building on the hypothesis that intermittent exposure to a HFHS diet during the juvenile developmental phase alters cognitive control and neurotransmitter systems within the brain, we sought to examine the effects of intermittent HFHS food consumption on social interaction and social memory in young rats. Spontaneous novel object recognition and odour recognition memory were examined to assess potential HFHS diet effects on long-term memory and olfaction. To highlight putative molecular pathways impacted by intermittent HFHS food consumption, we examined the expression of specific genes associated with neuroplasticity, monoamine signalling, and neuroinflammation in the PFC and hippocampus. Furthermore, we examined faecal microbiota composition to explore diet-induced alterations. Exploratory statistical analyses through linear modelling were performed to determine associations between faecal microbiota composition, behaviour and cortical gene expression.

2. Methods

2.1. Animals

Male (n = 32) albino Sprague Dawley rats (Animal Resources Centre, Western Australia) arrived at postnatal day (P)21 (mean body weight = ~50 g) and were housed in groups of four in a temperature (21 °C ± 2 °C; humidity 55 ± 5%) and light (12 h cycle lights on at 0700) controlled colony room. Standard laboratory rat chow (Meat Free Rat and Mouse Diet, Specialty Feeds, Western Australia; energy composition of 14 kJ/g; 23% protein, 12% fat, 65% carbohydrates) and water was available *ad libitum* throughout the experiment. Behavioural tests were performed between 0800 and 1400 and procedures were approved by the institution's Animal Care and Ethics Committee.

2.2. Diet administration

Rats were allocated to diet conditions: Control (normal rodent chow-fed, n = 8) or HFHS condition (n = 8). An additional age/weight matched cohort (n = 16) were allocated as sample animals for social memory and social interaction. Body weights were standardised in all treatment groups prior to the commencement of the diet (control: 75.5 ± 2.0g; HFHS: 76.4 ± 2.0 g), and rats were habituated to handling by the experimenters for seven days prior to commencing diet manipulations. Group-housing was used to negate confounding effects of social isolation stress [28]. Rats in the HFHS diet condition were provided with 2 h daily homecage access (between 0900-1100) to semi-pure HFHS pellets (Specialty Feeds, Western Australia, SP04-025; 18.4kJ/g digestible energy; composed of 20% fat (lard), 39.6% sucrose, 19.4% protein, providing 36% energy from lipids and 55% from sucrose), in addition to *ad libitum* standard chow and water access. Consumption of HFHS diet was calculated in the 2 h diet access period. Body weight was recorded at baseline before the diet began, and thereafter twice per week. Total 24 h energy intake per cage of four rats was calculated by measuring chow consumption and HFHS diet consumption as mass difference twice a week [29].

2.3. Behavioural analysis

A timeline of the general experimental procedures is presented in Figure 1A. Diet administration began on P28, coinciding the commencement of adolescence in male rats [13]. Behavioural tests were conducted in a room illuminated at 30 Lux, and sessions were recorded with a ceiling-mounted video camera. Social interaction, social memory, social odour preference, novel object recognition and odour recognition memory was assessed. Behaviours were scored by an observer who was blind to the group allocations using ODLog (v2.7, Macropod Software, Australia).

2.3.1. *Social interaction*

Social interaction tests were conducted in a square test arena (dimensions: 50 cm [length] x 50 cm [width] x 60 cm [height]) constructed from black Perspex. All rats were habituated to the arena 24 h prior to testing by being placed individually into the arena for 10 minutes.

Rats were held in individual cages for 15 minutes prior to social interaction testing. In the social interaction test, one rat from either the control or HFHS diet condition rat was placed in the arena with an unfamiliar partner matched for body weight (+/- 10g). To differentiate between animals, one rat was marked on its back with a black odourless fabric pen marker 24 h prior to testing. Test session duration was 10 min. The two rats were placed into the test arena simultaneously facing each other in opposing corners. Rats in the HFHS diet condition were tested 1 h after access to the HFHS pellets (post), and 23 h after HFHS pellet access (pre), counterbalanced across days and animals. The arena was cleaned with 70% ethanol between testing sessions to eliminate residual odour cues.

As social behaviour in rats has been shown to depend on the playfulness of its partner, both animals in a sample pair were considered as one experimental unit [12]. Videos were scored to measure i) the total time (s) spent in social interaction; ii) frequency of social investigation behaviour (sniffing, licking, grooming); iii) frequency of social play behaviour (pinning, pouncing); and iv) frequency of aggressive-like behaviour (biting, boxing, overt physical harm).

2.3.2. *Social memory*

Social memory testing was performed immediately after HFHS consumption to reduce confounding effects of reduced social contact in the HFHS diet rats. Social memory tests were

149 conducted in a circular arena (dimensions: 100 cm diameter, 50 cm height) constructed from
150 grey Perspex. The arena contained two wire chambers with plastic bases (dimensions: 18 cm
151 [length] x 20 cm [width] x 22 cm [height]). The wires were interspaced 1 cm apart to allow the
152 test rat to interact with the sample rats without physical contact. Sample, control and HFHS
153 diet rats were habituated to the testing apparatus 24 h prior to testing by being placed
154 individually into the arena with the empty chambers for 10 minutes.
155
156 Social memory was tested in two phases (see Supplementary Figure 1A). In Phase 1,
157 rats were placed in the arena for 5 min with one sample rat in a chamber and the other chamber
158 left empty. Time exploring the chamber containing the sample rat *versus* the empty chamber
159 was used as a measure of sociability [30]. The experimental rat was then removed and placed
160 into individual holding cages for a 5 min inter-trial interval (ITI) period. In Phase 2, the arena
161 contained the original sample rat (familiar) in a chamber and the previously empty chamber
162 contained a novel rat. The experimental rat was returned to the arena to explore for a 3 min
163 period. Between test phases the arena was cleaned with 70% ethanol to eliminate odour cues.
164
165 Videos were scored to measure the duration of time the rat spent exploring the chambers
166 during each phase. Sociability was quantified as the time spent exploring the chamber
167 containing the sample rat as opposed to the empty chamber, and social recognition memory
168 was measured as the time spent in proximity to the chamber containing the novel rat versus the
169 familiar sample rat.
170
171 *2.3.3. Social odour preference*
172 The wire chambers used for social recognition were either filled with soiled bedding from a
173 cage of young male rats (~5 weeks of age) housed in an adjacent holding room, or clean corn

cob bedding. Rats were allowed to freely explore the arena for 5 min and the amount of time spent exploring empty chambers containing either soiled or clean bedding was videoed and then scored by an experimenter.

2.3.4. Odour memory

Odour memory was conducted in the square test arena (as described in 2.3.1). Identical cylindrical stainless-steel containers (10 cm [height] x 6 cm [width]) with perforated stainless-steel lids were filled with corn cob bedding and then scented with 3 mL of peppermint or almond extract (Queen, Australia) to serve as odour stimuli (see Supplementary Figure 1B). The odour memory test consisted of 2 phases: a 5 min sample and 3 min test. During the sample phase two of the same scented containers were placed in opposite corners of the arena, and the rat was allowed to explore. The rat was then removed from the arena and placed in a holding cage for a 5 min retention period. The arena was thoroughly cleaned with 70% ethanol and one of the scented containers was replaced with an identical container filled with a novel odour for the test phase. Videoed behaviour was assessed for the duration of time the rat spent exploring each of the odour containers during each phase.

2.3.5. Object recognition memory

Object recognition (Supplementary Figure 1C) was conducted in the square test arena (as described in 2.3.1). Commercial objects (*e.g.* plastic bottles and tin cans) were used with differing heights (16-24 cm) and widths (7-14 cm). Rats explored two identical sample objects in the arena (sample phase; 5 min). The following day, 24 h after the sample phase, rats were tested for recognition of a familiar *versus* a novel object (test phase; 3 mins). The time the rat spent exploring each object during each phase was measured.

2.4. Sample collection

Following 28 days of diet access, rats were sacrificed prior to receiving the HFHS diet. Rats were anaesthetised with sodium pentobarbital (100 mg/kg i.p.), brains removed and the PFC and hippocampus (composed of dorsal and ventral poles) dissected and snap frozen in liquid nitrogen and stored at -80˚C for subsequent analysis by RT-PCR. Retroperitoneal and gonadal white adipose tissues (rpWAT; gnWAT) were dissected and weighed. Livers were weighed and visually scored for markers of hepatic steatosis based on previous criteria [31]. One faecal bolus was collected from the terminal caecum, snap frozen and stored at -80°C for later microbiota analysis.

2.5. ***Quantitative RT-PCR***

RNA was extracted using Tri-Reagent (Sigma-Aldrich) and RNeasy Mini kit (Qiagen), and quantity and purity of RNA was determined by UV/Vis spectroscopy (Nanodrop; Thermo-Fisher Scientific). RNA was converted to cDNA using a RT^2 First Strand Kit (Qiagen). Gene expression was quantified by Custom RT^2 Profiler PCR Arrays (Qiagen) with RT^2 SYBR Green Mastermix (Qiagen, Australia), and RT-PCR was then performed using a QuantStudioTM 7 Flex Real-Time PCR System (Applied Biosystems). Target genes were NLR family, pyrin domain containing 3 (*Nlrp3*), glutamate decarboxylase 1 (*Gad1*), brain-derived neurotrophic factor (*Bdnf*), dopamine receptor D1 (*Drd1*), dopamine receptor D2 (*Drd2*), monoamine oxidase A (*Maoa*), catechol-O-methyltransferase (*Comt*), 5-hydroxytryptamine (serotonin) receptor 4, G-coupled (*Htr4*), tumour necrosis factor alpha (*Tnf-α*), interleukin 6 (*Il6)*, and integrin, alpha M (*Itgam*) (all reagents from Qiagen; see Supplementary Table 1 for reference sequences). Analysis of relative gene expression was normalised to the housekeeping gene beta actin (*Actb*) using the $\Delta\Delta C_T$ method [32].

2.6. 16S rRNA gene amplicon sequencing and bioinformatics

Total DNA was isolated using the Bioline ISOLATE Faecal DNA Kit (Bioline). PCR was performed using Q5 DNA polymerase (New England Biolabs) with a primer set selected to amplify V3-V4 region of 16S rRNA gene (forward: ACTCCTACGGGAGGCAGCAG and reverse: GGACTACHVGGGTWTCTAAT). Sequencing was performed on an Illumina MiSeq instrument (2 × 300bp paired-end sequencing), following the method detailed by Fadrosh, Ma [33]. Sequences were joined in Quantitative Insights Into Microbial Ecology (QIIME) 1.9.1 (http://qiime.org) using the fastq-join method. Maximum-allowed percent differences within the overlapping region was zero. Sequences were de-multiplexed using the QIIME split library protocol, keeping only sequences with Phred quality score higher than 20. The dataset was inspected for chimeric sequences using Pintail [34]. Operational taxonomic units (OTUs) were clustered at 97% sequence identity using UCLUST [35] (min = 1443, max = 7082, median = 4466). Taxonomic assignments were performed against the GreenGenes database [36]. OTUs with a relative abundance of less than 0.01% were excluded.

2.7. ***Statistical analyses***

2.7.1. Behaviour, physiological parameters and brain mRNA expression

Results were analysed using repeat measures analysis of variance (ANOVA; body weight and energy intake), mixed design ANOVAs (social recognition memory, social interaction, sociability, novel odour recognition and novel object recognition), and one-way ANOVA (rpWAT, gnWAT, liver weight, RT-PCR values) with *post-hoc* Tukey and equality of error variance assessed, or multivariate linear models following significant correlations with *post-hoc* testing. $\Delta\Delta C_T$ values that exceeded ±2 standard deviations from the mean were excluded from analysis, resulting in group sizes of 6-8 per gene.

Social recognition memory and novel object recognition performance were converted to Exploration Ratios (Time[novel-familiar]/Time[novel+familiar]) to permit exploratory bivariate analysis using correlates (Pearson's *R*, one-tailed). This allowed the exploratory examination of associations between cortical mRNA expression across HFHS and control diet rats, and the performance of behaviours found to significantly differ between diet groups. Liver scores (mass and evidence of steatosis) were analysed using the Kruskal-Wallis test. Data were analysed with IBM SPSS Statistics 24, GraphPad Prism 7 and R.

2.7.2. Microbiota

Visualisation, alpha diversity and distance measures of microbiota were performed using the R packages *phyloseq, vegan* and *MixOmics.* Data were total-sum scaled (*i.e.* relative abundance of OTUs) and centre-log ratio transformed where appropriate [37]. Permutational ANOVA (PERMANOVA) of Bray-Curtis dissimilarity index was conducted with 999 permutations. The *DESeq2* package was used to undertake differential abundance testing [38], and multivariate analysis of variance (MANOVA) was used to test associations between *Firmicutes* to *Bacteroidetes* (FB) ratio, behaviour, and gene expression.

Significance for differential abundance analyses was assessed on the basis of a threshold *q*-value of 0.05 (*i.e. p*-value adjusted using the False Discovery Rate approach Benjamini, Drai [39]). Bivariate correlations were calculated using a two-tailed Pearson's *R* test.

3. Results

3.1. Body Weight, energy consumption and physiological measurements

Consistent with physical maturation during adolescence, all rats gained weight across the experiment, however HFHS diet rats showed a significantly greater increase in body mass than controls (time × diet group $F_{(8,112)} = 5.07$, $p < 0.001$; Figure 1B). Overall, rats consumed increasing amounts of energy across the four-week experimental period ($F_{(3,18)} = 81.4$, $p < 0.001$), and HFHS diet rats consumed more energy than control rats (diet group × time $F_{(1,6)} = 10.8$, $p < 0.001$; Figure 1C). At the experimental end point, HFHS diet rats had a greater body mass ($F_{(1,14)} = 4.516$, $p < 0.05$), rpWAT ($F_{(1,14)} = 5.54$, $p < 0.05$), gnWAT ($F_{(1,14)} = 4.71$, $p < 0.05$), and evidence of hepatopathology ($U = 5$, $p < 0.01$; Supplementary Table 2).

--- Figure 1 here ---

3.2. Effect of HFHS diet on social interaction before and after HFHS feeding

To assess the effect of HFHS diet consumption on social behaviour, we examined the total social exploration time one-hour prior to (*pre*) or following (*post*) HFHS food access. Social interaction duration during each test session did not differ in the standard chow fed control animals. However, HFHS diet rats spent less time engaged in social interaction pre-HFHS food access, compared to post-HFHS food access (diet access × diet group $F_{(1,14)} = 5.66$, $p < 0.05$; effect of diet group *pre* $F_{(1,14)} = 9.271$, $p < 0.01$, but not *post* $F < 1$; Figure 2A). Social investigation frequency was increased in the HFHS rats post-consumption (diet access × diet group $F_{(1,14)} = 8.6$, $p < 0.05$; HFHS $F_{(1,14)} = 21.59$, $p < 0.001$, control $F < 1$; Figure 2B). No significant differences were observed in the frequency of social play behaviours (Figure 2C), and no aggressive behaviours were observed. Together, this data suggests that social motivation is decreased in rats that consume intermittent HFHS diet when they have not had access to the palatable HFHS food for a 23-hour period.

3.3. Effect of HFHS diets on social recognition memory

Social behaviour has been typically examined in mice using the 'three-chamber' social approach test. We adapted this protocol for use in rats to examine whether changes in social recognition memory was altered by HFHS diet consumption. During the social approach phase of the sociability test (Figure 2D) both control and HFHS rats preferentially explored the novel 'sample' rat compared to the empty cage ($F_{(1,14)}$ = 275.5, p < 0.001) with no significant differences between groups or interaction effects (F_s < 1). However, social recognition was impaired in HFHS rats, which explored the familiar and novel rat equally, contrasting to the strong preference of control rats to explore the novel rat (chamber × diet group $F_{(1,14)}$ = 39.15, p < 0.001; control $F_{(1,14)}$ = 109.3, p < 0.001; HFHS $F_{(1,14)}$ = 2.6, p = 0.13; Figure 2E). Exploration ratios calculated from the test data (control = 0.80 ± 0.03; HFHS = 0.56 ± 0.03; as mean ± SEM) differed significantly between groups ($F_{(1,14)}$ = 33.2, p < 0.001).

3.4. No effect of diet on social odour preference or odour recognition memory

To confirm that the lack of social recognition memory in the HFHS rats was not due to a lack of olfactory sensitivity, we tested their ability to discriminate between clean and soiled bedding and between two non-social odours. Control and HFHS diet rats preferentially explored the chamber containing a social odour ($F_{(1,14)}$ = 217.8, p < 0.001; Figure 2F).

During odour recognition testing, control and HFHS diet rats showed no group or odour preference during the sample phase (no main effect of odour F < 1, diet group $F_{(1,3)}$ = 3.7, p = 0.15, odour × diet group $F_{(1,3)}$ = 3.4, p = 0.16; Figure 2G). At the time of testing, both control and HFHS rats preferentially explored the novel odour container, demonstrating odour recognition memory (odour × diet group $F_{(1,14)}$ = 3.0, p = 0.11; Figure 2H). Together, these results indicated that HFHS rats were unimpaired in odour discrimination, implying that the social recognition impairment (described in section 3.3) was not due to a lack of sensitivity to olfactory cues.

3.5. Effects of HFHS diet on novel object recognition

HFHS diet rats were tested on their ability to explore novel compared to previously explored objects. Control rats showed intact object recognition memory by preferentially exploring the novel object; though HFHS rats explored the familiar and novel objects equally, indicating impaired object recognition (object × diet group, $F_{(1,14)} = 50.7$, $p < 0.001$; control $F_{(1,14)} = 120.5$, $p < 0.001$; HFHS $F < 1$; Figure 2I). Exploration ratios calculated from the test data (control = 0.73 ± 0.01; HFHS = 0.52 ± 0.02) differed significantly between groups ($F_{(1,14)} = 60.8$, $p < 0.001$).

--- Figure 2 here ---

3.6. Diet effects on PFC and hippocampal mRNA expression

To determine whether short, intermittent periods of exposure to HFHS diet changed gene expression within the hippocampus and mPFC, we quantified mRNA expression of genes related to neuroplasticity, dopamine and monoamine signalling and neuroinflammation (Table 1). We found the majority of transcript changes occurred in the PFC. Compared to controls, the HFHS diet fed rats had reduced *Maoa* expression in the PFC ($F_{(1,13)} = 8.50$, $p < 0.05$) and hippocampus ($F_{(1,14)} = 6.89$, $p < 0.05$); *Comt* expression was significantly reduced in the PFC ($F_{(1,14)} = 19.0$, $p < 0.001$), as was PFC *Bdnf* was in HFHS consuming rats ($F_{(1,13)} = 4.99$, $p < 0.05$).

---- Table 1 here ----

3.7. Microbiota composition and analysis

The relative abundance of a number of specific taxa differed significantly between the two diet groups as shown by DESEq2 analysis (Figure 3A, Supplementary Table 3). HFHS diet increased levels of bacteria from *Firmicutes* phylum *Clostridales* family, including *Lachnospiraceae* (genus *Blautia,* $q < 0.04$; unspecified genus $q < 0.03$), *Ruminoccoceae* (genus unspecified $q < 0.01$) and *Veillonellaceae* (genus *Phascolarctobacterium* $q < 0.02$). HFHS diet increased bacteria from *Actinobacteria* phylum, family *Bifidobacteriaceae* (genus *Bifidobacterium,* $q < 0.04$), *Bacteroidetes* phylum, order *Bacteroidales* (unspecified genus $q < 0.05$) and *Tenericutes* phylum, order *Erysipelotrichaceae* (genus *Allobaculum* $q < 0.05$).

Alpha diversity did not differ between the HFHS and control groups measured by observed species, Chao 1, Shannon or Simpson indices (see Figure 3B; $Fs < 1$). Although there was visual overlap apparent on multidimensional scaling of the Bray-Curtis dissimilarity index (Figure 3C), PERMANOVA revealed significant dissimilarity on the basis of diet group ($R^2 = 0.18$, $p < 0.01$). PERMDISP2 revealed no significant heterogeneity of variances between the two groups ($p = 0.39$). Partial least squares discriminant analysis (PLS-DA), a linear classification model, identified the two components that discriminate maximally between the HFHS and control diet groups, showing a large proportion of variance accounted for by the first component (21%) and a lesser degree by the second (8%; Figure 3D).

---- Figure 3 here ----

3.8. Associations between diet effects, behavioural performance and gene expression

Correlations were performed between behaviours that differed between diet groups (social interaction pre-consumption of diet, social recognition and novel object recognition) and

biological measurements (WAT, bodyweight; and cortical gene expression). A number of significant associations were observed, in particular positive correlations between PFC expression of *Maoa* and social interaction pre-HFHS diet and object memory.

---- Figure 4 here ----

A number of bivariate correlations between physiological parameters (WAT and bodyweight) and gene expression were significant (Figure 4A and B). In particular, PFC and hippocampal Itgam expression was positively correlated with WAT (PFC: R^2=0.52, $p < 0.05$, HPC: $R^2 = 0.66$, $p < 0.01$) and bodyweight (HPC: $R^2 = 0.67$, $p < 0.01$), and hippocampal *Maoa* expression was negatively correlated with WAT ($R^2 = -0.45$, $p < 0.05$). Correlations between physiological parameters (WAT and bodyweight) and behavioural performance were observed (Figure 4C), in particular significant negative correlations between WAT and social recognition memory ($R^2 = -0.56$, $p < 0.05$), social interaction pre-HFHS diet ($R^2 = -0.58$, $p < 0.01$) and novel object recognition performance ($R^2 = -0.65$, $p < 0.01$).

Total WAT was significantly associated with PFC gene expression ($F_{(1,12)} = 5.4$, $p < 0.05$); specifically *Tnf-a* (adjusted $R^2 = 0.41$, $p < 0.01$), *Comt* (adjusted $R^2 = 0.23$, $p < 0.05$), *Maoa* (adjusted $R^2 = 0.29$, $p < 0.05$), and *Bdnf* (adjusted $R^2 = 0.74$, $p < 0.001$). A number of bivariate correlations between bodyweight and gene expression were significant (Figure 4A and A) however these associations did not persist in multivariate linear modelling (overall model $F_{(1,12)} = 2.1$, $p = 0.17$). There were no significant associations between hippocampal gene expression and body weight ($F < 1$). WAT weight predicted *Il6* expression in the hippocampus ($F_{(1,13)} = 4.86$, $p < 0.05$).

Associations between hippocampal and PFC genes differentially expressed in control and HFHS groups (see Table 1, Figure 4) and behavioural performance were examined. No predictive relationships were observed between PFC *Bdnf*, *Comt* or *Maoa* expression and social interaction pre-diet consumption, social memory or novel object recognition ($p = 0.17$; $p = 0.09$; $p = 0.16$ for overall model of each gene respectively). There was no evidence for a predictive relationship between hippocampal *Maoa* expression and behaviours ($p = 0.35$).

3.9. Associations between gut microbiota composition and social behaviour

Scores on pre-diet social behaviour, social recognition memory and novel object recognition tasks respectively were all significantly associated with the relative abundance of a number of bacterial taxa (all associations where $q < 0.05$ presented in Table 2). Social memory performance was associated with a large number of taxa. Higher social memory scores were associated with a greater abundance of bacteria from the *Bifidobacteriales* and *Bacteroidales* order, *Lachnospiraceae* family (*Blautia* and multiple unspecified genera), *Ruminococcaceae* family and genus *Allobaculum*. Novel object recognition was negatively associated with abundance of *Bacteroidales* and a number of taxa from the *Lachnospiraceae* family. Only three taxa were significantly associated with social behaviour pre HFHS diet: a relative reduction of *Bifidobacteriales* order and two unspecified genera from the *Lachnospiraceae* family.

----- Table 2 here -----

3.10. Firmicutes to Bacteroidetes ratio

There were no significant differences between the diet groups on *Firmicutes* to *Bacteroidetes* ratio (FB ratio; $t_{(9.63)} = -1.03$, $p = 0.33$). Samples were pooled across diet groups for subsequent FB ratio analyses, with diet group included to control for potential interaction effects.

Multivariate linear modelling demonstrated a significant relationship between FB ratio and the three behavioural dependent variables: social memory, novel object recognition and pre-diet social interaction ($F_{(3,11)} = 5.26$, $p < 0.05$). *Post-hoc* tests demonstrated strong evidence that FB ratio negatively predicted pre-diet social behaviour ($F_{(2,13)} = 11.46$, $p < 0.001$), but not object or social recognition memory.

3.11. Associations between gut microbiota and hippocampal and PFC gene expression

The hippocampal and PFC genes found to differ in expression between the control and HFHS diet groups (PFC: *Bdnf*, *Maoa*, *Comt*; hippocampus: *Maoa*, $p_s < 0.05$) were tested for their associations with differential abundance of bacterial taxa. Of these, significantly differentially abundant taxa ($q < 0.05$) were apparent only for *Maoa* (Table 3). PFC *Maoa* expression was positively associated with one genus of the *Lachnospiraceae* family, whilst a number of bacteria across the four primary phyla were differentially abundant on the basis of hippocampal *Maoa* expression in both positive and negative directions.

----- Table 3 here -----

4. Discussion

The data presented in this study shows that daily intermittent consumption of a HFHS diet during adolescence leads to deficits in social interaction and social memory, and impaired object recognition memory in rats. This study also demonstrated associations between diet-induced alterations to social behaviour with microbiota and changes in gene expression associated with reward pathways and neuroplasticity.

We observed that the effects of HFHS diet on social interaction were limited to immediately prior to ingestion when rats had not consumed HFHS pellets for 23 h, though not after access to HFHS foodstuffs. Based on decreased expression of *Maoa* and *Comt* genes that regulate catecholamine metabolism, we postulate that a junk food mimetic diet can lead to altered monoamine neurotransmission and a resultant increase in anxiety-like behaviour. Thus, intermittent access to a HFHS diet may influence social interaction, as comparable interaction durations were observed following access to a diet rich in fats and sugars. Moreover, social interaction frequency was significantly increased after rats had access to the HFHS food, suggesting that the rewarding aspects of social interaction may have been amplified following ingestion of a diet modelled on obesity-associated nutritional intake, and that recent HFHS diet consumption may also reduce anxiety.

Social play is important for neurobehavioural development and is also intrinsically linked to proliferation of neurotransmitter pathways, with the dopaminergic mesolimbic system playing a major role in normal social interactions [40]. We observed no differences in frequencies of social play behaviours between diet groups, though these data should be interpreted with some caution. The group housing conditions and brief period of isolation used prior to behavioural testing may have obscured subtle variations between groups as social isolation amplifies subsequent social play behaviour [8]. Another possible explanation is that social play activities tend to decline as adolescence progresses, and that the lack of measurable differences could be attributed to the age of test animals representing mid-to-late adolescence [12]. Extended studies focusing on both dietary habits in early adolescence and potential delayed or enduring long-term effects into adulthood are needed to assess whether poor nutrition reflected by a HFHS diet are associated with potential critical windows of susceptibility representing social behavioural changes.

Social recognition performance differed between control and HFHS rats, with rats exposed to the dietary intervention demonstrating no preference for the test chamber containing the novel rat during the test phase. This is supported by a recent study showing that acute exposure to a high fat diet in juvenile rats impaired social memory [10]. As rats showed differences in their duration of time engaged in social interaction prior to consuming the HFHS food, the social memory testing was conducted following HFHS access to ensure that any memory deficits observed were not due to reduced social contact in the treated animals. Initial sociability during the sample phase did not differ between HFHS and control diet rats, indicating that social memory was impacted specifically by the diet constituents. Social memory has been shown to depend upon both PFC and hippocampal function [18, 41], and our measured alterations to markers of monoamine neurotransmission and neuroplasticity may underlie the observed social changes. This is also complemented by impaired long term novel object recognition, which is also associated with hippocampal dysfunction [42]. Moreover, both HFHS and control diet rats showed preference for a social odour and showed intact odour recognition memory. Thus, intermittent HFHS diet did not impact olfactory discrimination, and indicates that social memory deficits are not associated with impaired olfactory function.

With respect to the observed variations in mRNA expression of enzymes *Maoa* and *Comt* in the PFC, it is highly plausible that a HFHS diet adversely affects neurotransmitter activity, specifically dopamine, that is integral to social behaviour and cognition. Dopamine has a primary role in the corticolimbic circuitry involved in the regulation of food reward [43]. By mediating deamination of dopamine, monoamine oxidase activity has a key role in controlling the availability of cortical dopamine and also functions in conjunction with catecholamine-*O*-methyltransferase in dopamine breakdown and excretion as inactive

493 homovanillic acid. Changes to monoamine signalling may underpin the altered social
494 behaviour and social memory observed in HFHS diet rats, supported by reports of diet-induced
495 alterations to dopamine receptor expression in the striatum [44]. With no measurable change
496 in dopamine receptor (*Drd1a*/*Drd2*) expression in the hippocampus or PFC, it is suggestive
497 that impaired catecholamine metabolism, rather than reuptake is the major driver of
498 behavioural changes related to dopamine following obesogenic diet consumption [6, 45].
499 Further studies should examine whether other reward associated genes, such as serotonin and
500 μ-opioid receptors are altered by this diet protocol, and also the involvement of oxytocin
501 signalling mechanisms [10].
502
503 Reduced PFC *Bdnf* expression was observed in HFHS consuming rats, which also
504 correlated positively with novel object recognition performance. This diet-induced change may
505 reinforce the changes to social behaviours and cognition as BDNF signalling has a critical role
506 in memory encoding [46]. Decreased levels of BDNF observed in the hypothalamus, PFC,
507 and/or serum have been shown to correlate with mood disorder-like behaviours in animals and
508 humans [47] and high fat diet consumption reduces hippocampal BDNF levels [48, 49] linking
509 BDNF to emotional processes. Gut microbiota composition may influence cortical BDNF, as
510 demonstrated by previous studies indicating reduced cortical and hippocampal *Bdnf* gene
511 expression in GF mice [50], and antibiotic-induced microbiota dysbiosis altered protein levels
512 of BDNF in the amygdala and hippocampus as well as reduced anxiety-like behaviours in the
513 light-dark box [51]. Thus, microbiome influences on BDNF may be a critical factor in
514 cognition and emotional regulation.
515
516 Excessive consumption of saturated fats has been shown to induce secretion of pro-
517 inflammatory cytokines by adipocytes and macrophages, and affect the integrity of the blood-

brain barrier [52], allowing pro-inflammatory cytokines and immune-response cells to reach the brain [53]. Interestingly, no significant changes between groups were observed in inflammatory marker mRNA expression (*Il6*, *TNF-α*, *Nlrp3*, *Itgam*), and trends indicated that PFC expression of *Il6* and *Nlrp3* were lower in HFHS diet rats. This may be due to the age of the rats, as emergent evidence suggests that the modulatory effects of obesogenic diets on inflammatory markers occur in an age-dependent manner, with younger rats showing resistance to neuroinflammation [9]. However, *Itgam* (also called cluster of differentiation molecule 11b, or CD11b) expression in the PFC and hippocampus positively correlated with WAT, indicative that increased adiposity was associated with aspects of neuroinflammation [54]. Moreover, evidence indicates that obesity-induced neuroinflammation is dependent on the type of diet in terms of fat and sugar content, the duration of the diet, and regional differences in brain structures [55]. Future studies utilising immunohistochemistry to examine microglia morphology and astrogliosis are needed to validate the region-specific impact of obesogenic diets on neuroinflammatory effects.

The effects of obesogenic high fat, high sugar and Western diets on the gut microbiome have been extensively studied in rodents, with typical observations including the altered abundance of the *Bacteriodetes* and *Firmicutes* phyla [56-59]. Notably, *Bacteroites* (order *Bacteroidales)* phyla were increased in HFHS diet-fed rats. This contrasts other studies and indicates that not all the members of the *Bacteriodetes* family are decreased with adiposity. Whilst we did not observe an overall shift in the FB ratio, the data presented here suggests that intermittent HFHS diet protocol significantly altered the gut microbiota signature, and supports the concept that a phylum-wide binary distinction does not sufficiently reflect the complexity of diet-induced changes to the gut microbiome as suggested in previous reports [60, 61]. FB

ratio changes may therefore become more prominent with more chronic hypercaloric feeding schedules, and the development of pronounced obesity.

Moreover, our detected increase in the abundance of the *Firmicutes* family *Ruminococcaceae* is consistent with previous studies that found these taxa to be increased in mice [62] and rats [63] consuming a high fat diet. Taxa from *Lachnospiraceae* and *Ruminoccoceae* families of the *Clostridiales* order were the most common bacterial predictors of social behaviour and recognition memory, converging with clinical studies that show alterations in microbiome populations in neuropsychiatric disorders including major depressive disorder and autism [64, 65]. Social avoidance behaviour in non-obese diabetic mice has been associated with increased abundance of *Lachnospiraceae*, *Ruminococcaceae* and *Clostridiales*, and the transfer of intestinal microbiota from these mice to microbiota-depleted recipients evoked similar behavioural phenotypes [66]. As such, our observations converge with evidence indicating the influence of diet on social behaviours *via* the gut-brain-microbiota axis [67, 68].

Further studies using faecal transplants from HFHS diet animals are necessary to elucidate the mechanisms underpinning the neural effects of gut microbiome. Our study was limited by faecal samples being taken only at the experiment endpoint, and behaviour and microbiome analyses may have been more powerful if taken from the same time points. Moreover, a direct comparison between HFHS diet effects and behaviour could be made if an additional group that received ad libitum access to the HFHS was included in the study. In addition, these animals could further serve as an additional control as behaviour around the HFHS diet access period to be likely changed due to conditioning. Furthermore, as locomotor activity can be influenced by motivation, anxiety and body weight, and can by itself influence

the results of behavioural readouts, additional examination of locomotor behaviour in this study is warranted to further define diet induced alterations to social behaviour.

The results presented here support the need for further studies including generating metagenomic predictions from the bacterial communities shed light onto the metabolic pathways impacted by intermittent HFHS diet consumption. Modulation of the gut-brain axis dynamics has clinical implications for neuropsychiatric conditions, and emerging 'psychobiotic' treatment strategies that have been indicated to ameliorate depressive [69] and anxiety-like behaviours [51] in mice, such as increased hippocampal *Bdnf* expression resulting from prebiotic administration [70]. As such, harnessing the microbiome may provide a route for the attenuation of diet and obesity evoked cognitive and emotional alterations.

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780 **Tables**

781 **Table 1.** The effects of intermittent high fat and high sucrose (HFHS) diet exposure on
782 prefrontal cortex and hippocampal gene expression, mean (±SEM), * = $P<0.05$, **=$P<0.01$, #
783 = $P<0.10$

784

785 **Table 2.** Associations between relative abundance of taxa in faecal microbiota and behavioural
786 outcomes ($q<0.05$).

787

788 **Table 3.** Associations between relative abundance of taxa in faecal microbiota and gene
789 expression ($q<0.05$).

790 **Table 1.** The effects of intermittent high fat and high sucrose (HFHS) diet exposure on
791 prefrontal cortex and hippocampal gene expression. Table shows Mean (SEM), * = $P<0.05$,
792 **=$P<0.01$, # = $P<0.10$

Gene	Prefrontal cortex			Hippocampus		
	Control	**HFHS**	***p*-value**	**Control**	**HFHS**	***p*-value**
Neuroplasticity						
Gad1	0.84 (0.04)	0.94 (0.07)	*n.s.*	1.00 (0.08)	0.94 (0.06)	*n.s.*
Bdnf	1.00 (0.10)	0.72 (0.03)*	*0.045*	1.00 (0.08)	0.96 (0.17)	*n.s.*
Dopamine receptors						
Drd1a	1.00 (0.23)	0.65 (0.13)	*n.s.*	1.00 (0.11)	0.95 (0.09)	*n.s*
Drd2	1.00 (0.32)	0.56 (0.15)	*n.s.*	0.93 (0.03)	0.85 (0.07)	*n.s.*
Monoamine synthesis						
Maoa	1.00 (0.04)	0.86 (0.02)*	*0.012*	1.00 (0.04)	0.83 (0.05)*	*0.02*
Comt	1.00 (0.02)	0.83 (0.03)**	*0.001*	1.00 (0.07)	1.08 (0.09)	*n.s.*
Serotonin receptor						
Htr4	0.85 (0.07)	0.79 (0.08)	*n.s.*	1.00 (0.05)	0.96 (0.04)	*n.s.*
Inflammation						
Tnf-α	1.00 (0.16)	0.79 (0.11)	*n.s.*	1.00 (0.21)	0.65 (0.06)	*n.s.*
Il6	1.00 (0.15)	0.62 (0.08)$^{\#}$	*0.060*	0.81 (0.14)	0.59 (0.11)	*n.s.*
Nlrp3	1.00 (0.06)	0.85 (0.03)$^{\#}$	*0.056*	1.00 (0.18)	1.03 (0.14)	*n.s.*
Itgam	1.00 (0.10)	1.09 (0.09)	*n.s.*	1.00 (0.11)	1.23 (0.19)	*n.s.*

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Table 2. Associations between relative abundance of taxa in faecal microbiota and behavioural outcomes (q<0.05).

					Social recognition memory		**Object recognition memory**		**Pre-diet social interaction**	
Phylum	**Class**	**Order**	**Family**	**Genus**	log2FoldChange	*q*	log2FoldChange	*q*	log2FoldChange	*q*
Actinobacteria	Actinobacteria	Bifidobacteriales	Unspecified	Unspecified	5.13	<0.01			-0.04	0.01
Firmicutes	Bacteroidia	Bacteroidales					-7.27	0.03		
	Clostridia	Clostridiales	Lachnospiraceae	Unspecified	8.51	<0.01				
					4.59	0.04				
					4.20	<0.01	-9.99	<0.01		
					-4.87	0.04				
					4.28	0.04				
					4.24	0.05				
					4.66	0.04				
					4.30	0.02				
				Blautia					-0.04	0.01
									-0.04	0.01
							-8.32	0.02		
							-7.89	0.03		
					4.72	0.01				
					4.48	0.04	-8.44	0.02		
					4.51	0.01				
					4.91	0.01				
					5.42	0.01				
				Unspecified			-6.90	0.04		
			Ruminococcaceae		6.06	<0.01				
					4.45	0.02				
					4.13	0.04				
Tenericutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Allobaculum	2.90	0.04				

796
797

Table 3. Associations between relative abundance of taxa in faecal microbiota and gene expression (q<0.05).

					Maoa Hippocampus		Maoa Prefrontal cortex	
Phylum	**Class**	**Order**	**Family**	**Genus**	log2Fold Change	*q*	log2Fold Change	*q*
Actinobacteria	Actinobacteria	Bifidobacteriales	Unspecified	Unspecified	-7.19	0.05		
					-7.87	0.01		
			Bifidobacteriaceae	Bifidobacterium	-8.18	0.01		
					-7.18	0.03		
					-7.07	0.03		
Bacteroidetes	Bacteroidia	Bacteroidales	Unspecified	Unspecified	-7.88	0.03		
			Rikenellaceae	Alistipes	10.40	<0.01		
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Unspecified	7.17	0.04		
							9.91	0.01
			Ruminococcaceae	Unspecified	8.43	0.01		
Tenericutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Allobaculum	-7.53	0.03		
					-8.32	0.02		
					-8.96	0.01		
					-8.37	0.01		

Figure Legends

Figure 1. **Experimental design and physiological effects of HFHS diet consumption.** A) Timeline of experimental procedures showing the ages of the rats at each behavioural test (conducted at the same age in all animals) and at sacrifice. Social interaction testing was conducted both before and after access to palatable HFHS food in the HFHS rats. As HFHS rats showed differences in social interaction pre HFHS food, social memory testing, and other behavioural tests were conducted after HFHS access, to ensure that any memory or behavioural deficits observed were not due to reduced social contact in the HFHS diet rats. B) Mean body weights of control and HFHS rats across the 4-week diet exposure period. C) Mean energy consumption (kJ) per cage of rats across the 4-week diet exposure period. Error bars represent +SEM. * indicates $P \leq 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 2. **Impact of HFHS diet consumption on behaviour.** Social behaviours between control / HFHS diet exposed rats and a novel weight / age matched conspecific. HFHS diet rats were tested either 23h after HFHS pellet access "pre" or 1h after access to the HFHS pellets "post", A) Total duration of social contact between rats, B) frequency of social interactions, and C) frequency of social play. Performance of HFHS diet and control rats in social recognition memory - D) exploration times of the chamber containing the sample rat "sample" and empty chamber "empty" during the sample phase of social memory testing, E) exploration times of the chamber containing the familiar sample rat and chamber containing a novel sample rat. F) Exploration time of chambers containing soiled bedding "social odour" or clean bedding. G) Exploration of the odours during the sample phase. H) Novel odour recognition performance in control and HFHS diet rats during the test phase following a 5 min delay. I) Novel object recognition performance during the test phase following a 24h delay**.** Error bars

represent +SEM. ** $P<0.01$. Error bars represent +SEM. * indicates $P\leq0.05$, ** $P<0.01$, *** $P<0.001$ between groups comparisons.

***Figure 3*. Impact of HFHS diet on faecal microbiota.** A) Graphical depiction of DESEq2 analysis. Each coloured circle represents one bacterial genus that was more abundant in the HFHS than control group (q <0.05). Log2 fold change refers to the difference abundance of the log2 values between diet groups for each bacterial genus. B) No significant differences in alpha diversity of faecal microbiota between HFHS and control diet groups. Each panel represents one alpha diversity measure as follows: Observed = total number of OTU's observed; Chao1 = richness estimator (estimate of the total number of OTU's present in a community); Shannon and Simpson = microbial indexes of diversity. Boxes span the first to third quartiles; the horizontal line inside the boxes represents the median. Whiskers extending vertically from the boxes indicate variability outside the upper and lower quartiles, and the single black circles indicate outliers (all $P>0.05$). C) Permutational ANOVA (PERMANOVA) of Bray-Curtis dissimilarity index revealed significant dissimilarity on the basis of diet group. D) Partial least-squares discriminant analysis (PLS-DA) Figure showing a large proportion of variance accounted for by the first component (21%) and a lesser degree by the second (8%). Each point represents a sample.

***Figure 4*. Correlations between behaviour, cortical gene expression and physiological effects of the HFHS diet.** Heatmap of bivariate correlations (Pearson's R^2) between the behavioural assays social recognition memory, social interaction and novel object recognition performance, and A) prefrontal cortex gene expression, B) hippocampal gene expression and C) physiological parameters. *=P<0.05, **=P<0.01

848

849

850

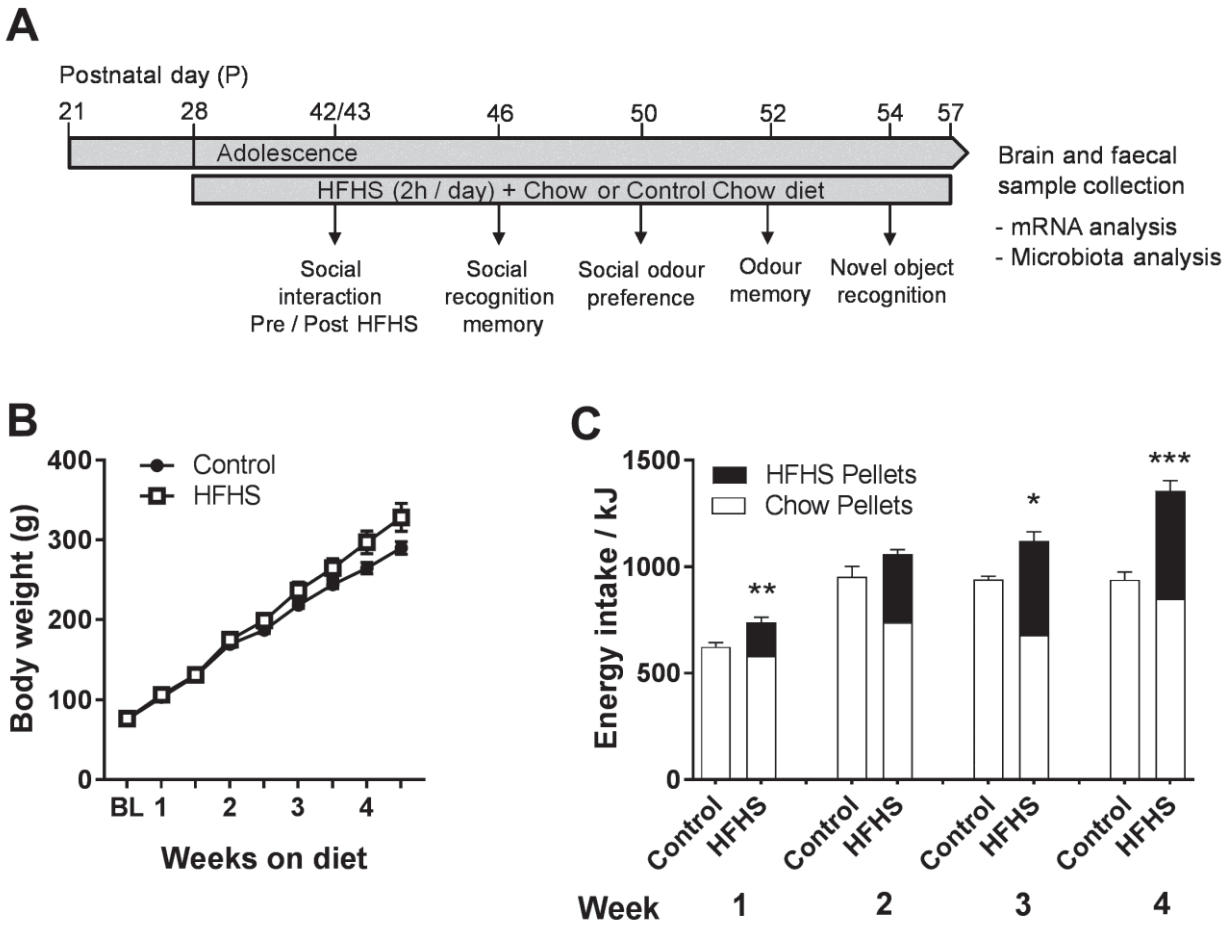


Figure 1

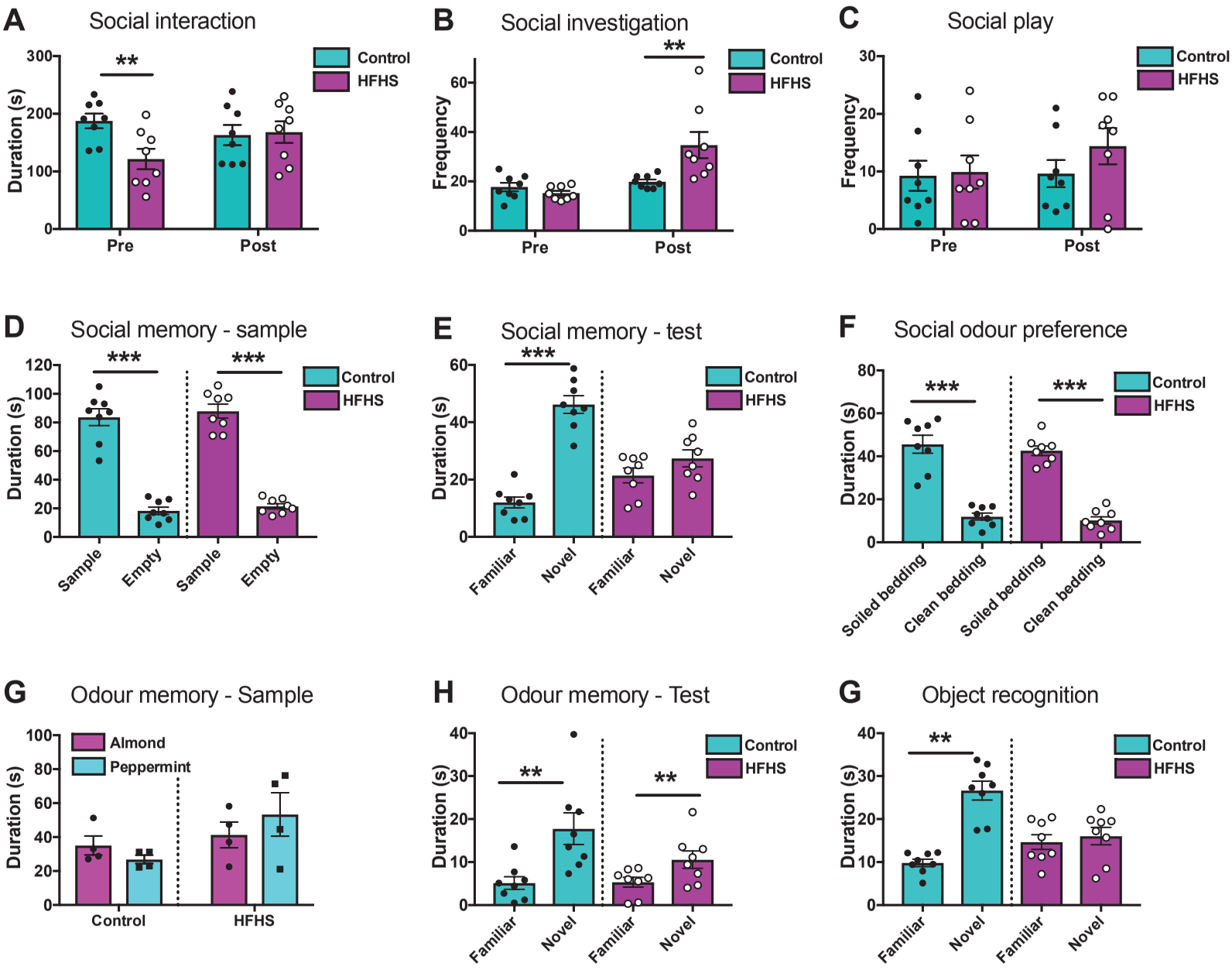

A
Social interaction
Duration (s)
300
200
100
0
**
Control
HFHS
Pre
Post
B
Social investigation
Frequency
60
40
20
0
**
Control
HFHS
Pre
Post
C
Social play
Frequency
30
20
10
0
Control
HFHS
Pre
Post
D
Social memory - sample
Duration (s)
120
100
80
60
40
20
0
***
***
Control
HFHS
Sample
Empty
Sample
Empty
E
Social memory - test
Duration (s)
60
40
20
0
***
Control
HFHS
Familiar
Novel
Familiar
Novel
F
Social odour preference
Duration (s)
80
60
40
20
0
***
***
Control
HFHS
Soiled bedding
Clean bedding
Soiled bedding
Clean bedding
G
Odour memory - Sample
Duration (s)
100
80
60
40
20
0
Almond
Peppermint
Control
HFHS
H
Odour memory - Test
Duration (s)
40
30
20
10
0
**
**
Control
HFHS
Familiar
Novel
Familiar
Novel
G
Object recognition
Duration (s)
40
30
20
10
0
**
Control
HFHS
Familiar
Novel
Familiar
Novel

Figure 2

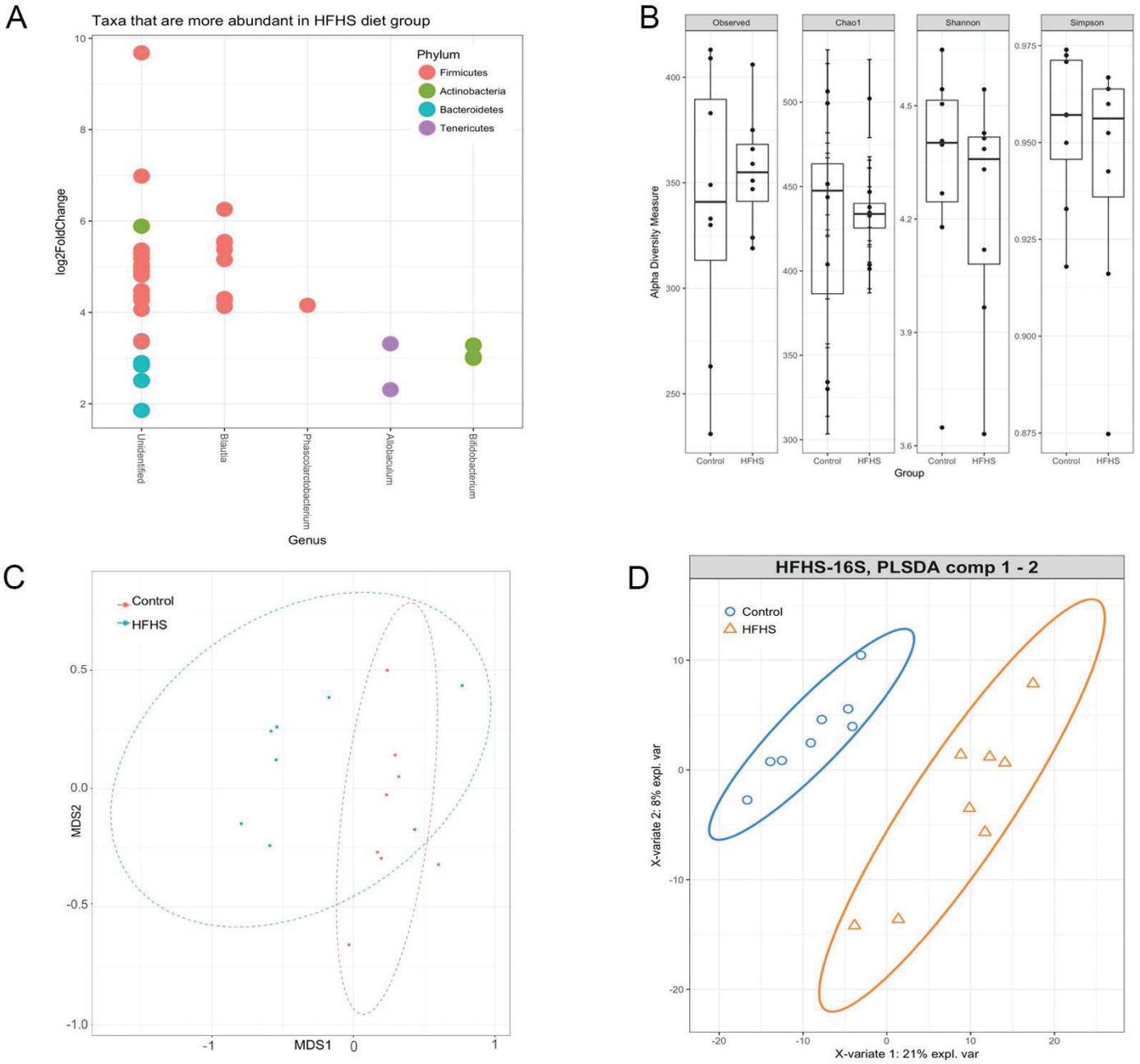

A
Taxa that are more abundant in HFHS diet group
Phylum
Firmicutes
Actinobacteria
Bacteroidetes
Tenericutes
log2FoldChange
Unidentified
Blautia
Phascolarctobacterium
Allobaculum
Bifidobacterium
Genus
B
Observed
Chao1
Shannon
Simpson
Alpha Diversity Measure
Control
HFHS
Group
C
Control
HFHS
MDS2
MDS1
D
HFHS-16S, PLSDA comp 1 - 2
Control
HFHS
X-variate 2: 8% expl. var
X-variate 1: 21% expl. var

Figure 3

a) Prefrontal Cortex

Gene	Social recognition memory	Social interaction pre-HFHS diet	Spontaneous object recognition	WAT	Body Weight
Nlrp3					
Gad1	*				
Bdnf			*		
Drd1a					
Drd2					
Maoa		*	*		
Comt	*	**	*		
Htr4					
Tnf					
Il6					
Itgam				*	

b) Hippocampus

Gene	Social recognition memory	Social interaction pre-HFHS diet	Spontaneous object recognition	WAT	Body Weight
Nlrp3					
Gad1					
Bdnf					
Drd1a					
Drd2					
Maoa	*		*	*	
Comt					
Htr4					
Tnf	**		*		
Il6					
Itgam				**	**

c) Physiological parameters

Parameter	Social recognition memory	Social interaction pre-HFHS diet	Spontaneous object recognition
WAT	*	**	**
Body Weight			*

Pearson R^2

0.6
0.3
0
-0.3
-0.6

Figure 4

Supplementary Information

Supplementary Table 1. Reference sequences of genes of interest

Gene	Gene symbol	Accession number
NLR family, pyrin domain containing 3	Nlrp3	NM_001191642
Glutamate decarboxylase 1	Gad1	NM_017007
Brain-derived neurotrophic factor	Bdnf	NM_012513
Dopamine receptor D1	Drd1a	NM_012546
Dopamine receptor D2	Drd2	NM_012547
Monoamine oxidase A	Maoa	XM_001058993, XM_343764
Catechol-O-methyltransferase	Comt	NM_012531
5-hydroxytryptamine receptor 4	Htr4	NM_012853
Tumour necrosis factor-alpha	Tnf	NM_012675
Interleukin 6	Il6	NM_012589
Integrin, alpha M	Itgam	NM_012711

Actin, beta	Actb	NM_031144

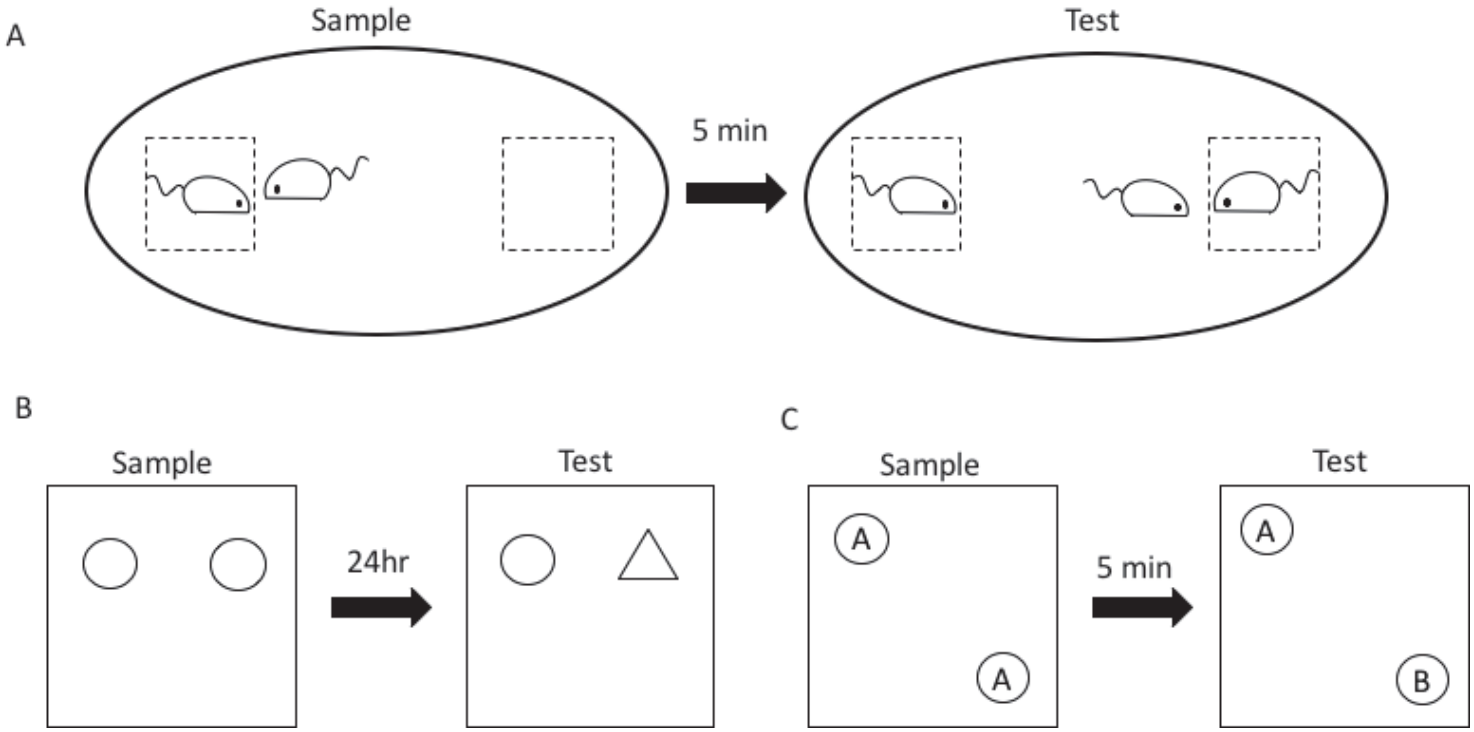
Supplementary Table 2. Effect of diet type on physiological parameters (mean ±SEM) measured after 28 days of diet exposure. * indicates $P \leq 0.05$.

Diet group	Body Weight /g	rpWAT /g	gnWAT /g	Liver score	Liver weight /g
Control	318.6 (8.3)	3.3 (0.4)	2.2 (0.2)	0	13.7 (0.5)
HFHS	366.4 (20.1)*	5.0 (0.6)*	3.6 (0.6)*	1*	16.5 (1.4)*

Supplementary Table 3. Analyses of differences in relative abundance differences in faecal microbiota of HFHS group relative to Control (q<0.05).

Phylum	Class	Order	*Family*	Genus	log2foldchange	*q*
Actinobacteria	Actinobacteria	Bifidobacteriales	Unspecified	Unspecified	5.89	<0.01
			Bifidobacteriaceae	Bifidobacterium	3.28	0.02
					3.03	0.04
					3.00	0.03
Bacteroidetes	Bacteroidia	Bacteroidales	Unspecified	Unspecified	2.84	<0.01
					3.35	0.01
					2.90	0.03
					3.38	0.05
					1.86	0.05
					2.51	0.05
Firmicutes	Clostridia	Clostridiales	Lachnospiraceae	Blautia	5.16	0.01
					4.30	0.04
					5.38	0.00
					5.53	0.00
					4.13	0.03
					6.26	0.00
					5.55	0.00
					4.28	0.04
				Unspecified	3.37	0.03
					4.28	0.02
					4.99	0.00
					5.03	0.01
					5.00	0.01

			Lachnospiraceae	Unspecified	5.37	0.01
					4.36	0.03
					4.81	0.05
			Ruminococcaceae	Unspecified	5.18	0.00
					4.90	0.01
					6.98	0.00
			Veillonellaceae	Phascolarctobacterium	4.16	0.02
			Unspecified	Unspecified	4.47	0.02
					4.07	0.04
					5.29	0.02
					9.68	0.00
Tenericutes	Erysipelotrichi	Erysipelotrichales	Erysipelotrichaceae	Allobaculum	2.31	0.04
					3.31	0.00



Supplementary Figure 1 A) Schematic of social memory testing procedure. B) Schematic of novel odour recognition procedure, where A and B are different odours contained in identical containers. C) Schematic of novel object recognition procedure.